Unit Title

Measuring Motivation and Reward-Related Decision Making in the Rodent Operant Touchscreen System

Author(s)

Christopher J. Heath[1,*], Benjamin Phillips[1], Timothy J. Bussey[1] and Lisa M. Saksida[1]

Contact information

[1]Department of Psychology and MRC/Wellcome Trust Behavioural and Clinical Neuroscience Institute, University of Cambridge, Downing Street, Cambridge, CB2 3EB, UK

Email: cjh201@cam.ac.uk

Phone: +44 (0) 1223 333588

ABSTRACT

This Unit is designed to facilitate implementation of the fixed and progressive ratio paradigms and the effort-related choice task in the rodent touchscreen apparatus to permit direct measurement of motivation and reward-related decision making in this equipment. These protocols have been optimized for use in the mouse and reliably yield stable performance levels that can be enhanced or suppressed by systemic pharmacological manipulation. Instructions are also provided for the adjustment of task parameters to permit use in mouse models of neurodegenerative disease. These tasks expand the utility of the rodent touchscreen apparatus beyond the currently available battery of cognitive assessment paradigms.

Keywords:

Touchscreen, motivation, effort-related choice, mouse

INTRODUCTION

The rodent touchscreen testing system is becoming widely used in neuroscience research laboratories. To date a large number of paradigms have been developed for this apparatus to examine a wide range of cognitive domains in both rats and mice (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). The protocols presented in this Unit expand the range of psychological constructs that can be assessed in this equipment to include motivation and reward-related decision making (Heath et al., In press).

Specifically, Basic Protocol 1 provides instructions for the initial preparation and training of mice to emit operant responses on a Fixed Ratio (FR) schedule in the touchscreen apparatus. Following stabilization of FR performance, animals can be streamed onto the Progressive Ratio (PR) schedule (see Basic Protocol 2) for assessment of motivation or the Effort-Related Choice (ERC) schedule (see Basic Protocol 3) for evaluation of reward-related decision making. It is possible to transfer animals from the PR to the ERC schedules (Heath et al., In press) as recommended by the battery approach previously suggested for this apparatus (Horner et al., 2013). Alternate Protocols are provided to allow mouse models of neurodegenerative disease to be assessed with the PR schedule.

Note: Protocols in this Unit involve live vertebrate animals. Approval for performance of these procedures must therefore be obtained from appropriate national/institutional regulators before implementation. Adherence to all required standards for the care and use of laboratory animals must be maintained throughout.

BASIC PROTOCOL 1

Initial handling, food restriction and operant chamber acclimatization

The rodent touchscreen apparatus explicitly avoids use of aversive stimuli to promote conditioning. Training is therefore dependent on appetitive reinforcement given in the form of palatable gustatory rewards. To promote reward consumption and therefore facilitate training, mild food restriction must be implemented. Following stabilization of restricted body weight, animals must be familiarized with the reward earned in the touchscreen apparatus and subsequently the apparatus itself. Once habituated to the behavioral equipment, mice are trained to emit the operant response required in both the PR (see Basic Protocol 2) and ERC (see Basic Protocol 3) schedules.

Materials

Rodents – Mice can be obtained from commercial suppliers (e.g. Jackson Laboratories, Taconic Biosciences, Charles River Laboratories). Otherwise, animals are bred in the laboratory or received from other appropriately licensed institutions. While mice of either sex can be tested in the touchscreen apparatus, we currently only examine males. This minimizes data variability induced by estrus-cycle mediated changes in behavioral performance and reduces conspecific aggression induced by testing both sexes in the same apparatus (Frick and Berger-Sweeney, 2001; Meziane et al., 2007). Mice of the C57BL/6 and 129 substrains are most commonly tested in the touchscreen apparatus though other strains can be readily examined. While aged animals can be tested in the touchscreen system (Creer et al., 2010) we find that training is greatly facilitated in young adults (approximately 10-14 weeks of age). The number of animals required for a given experiment is highly dependent on the objectives of the study and the manipulations planned (e.g. genetic vs. pharmacological vs. surgical). As far as possible, group sizes should be determined based on power calculations derived from analysis of previous studies using the same mouse strain.

Housing – Mice should be housed in groups with appropriate substrate, bedding, shelter and environmental enrichment (e.g. chew blocks) in an appropriate rodent housing facility maintained at a constant temperature (21 ± 2°C) and humidity (55 ± 10%) with a 12 hour light-dark cycle. We typically use an inverse light cycle (room lights off: 0700hrs) and train animals during the day (approximately 0800-1800hrs) to correspond with the most active part of their circadian cycle, though we have not observed significantly impaired performance in mice housed in standard light cycle conditions (room lights on: 0700hrs) tested at similar times of day (Beeler et al., 2006; Roedel et al., 2006; Chaudhury and Colwell, 2002).

Husbandry – All cages should be provided with drinking water throughout and (until food restriction begins) standard rodent food pellets (e.g. Diet RM 3, Special Diet Services, UK) and should be changed once weekly. To minimize disruption of performance, we recommend scheduling cage changes to occur on the same day each week and always after behavioral training has been completed on that day.

Rewards – Rodent touchscreen testing systems can be equipped with solid or liquid reward dispenser systems. We use 14mg Bio-Serv purified rodent Dustless Precision Pellets (Sandown Scientific, Esher, UK) or strawberry milkshake (Yazoo, FrieslandCampina, Horsham, UK). A single reward in all paradigms in this Unit is defined as 1 reward pellet or 20µL milkshake.

Rodent touchscreen system – This apparatus is now available from a number of commercial suppliers (e.g. Campden Instruments Ltd, Med Associates Inc.) or can be built in-house (Wolf et

al., 2014; Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). The protocols in this Unit have been optimized for use in the mouse touchscreen system produced by Campden Instruments and may require modification for use in other systems.

Briefly, the Campden Instruments touchscreen chamber consists of a trapezoidal behavioral arena with a touchscreen monitor (12.1 inch screen, 800 x 600 resolution) at one end and reward collection magazine at the other (20cm h x 18cm l x 6-24cm w (tapering from touchscreen to magazine)). The walls of the arena are black plastic, the lid is transparent plastic and the floor is perforated stainless steel beneath which a stainless steel waste tray is positioned. The entire assembly is housed in a dense fiberboard sound attenuating chamber equipped with a fan to provide low level background noise and air circulation. An LED houselight (not used in the protocols presented in this Unit), click/tone generator and a video camera are mounted above the arena adjacent to an electronic pellet dispenser. For liquid reward equipped chambers a peristaltic pump is sited centrally behind the touchscreen unit. An infra-red (IR) beam is used to detect entries in the magazine and two further beams are arrayed across the length of the arena to provide activity measurements (Figure 1). IR beams are also arrayed across the surface of the touchscreen such that animals do not have to exert direct pressure for a response to be registered. To minimize unintended screen touches and to demarcate screen response locations a black plastic mask is fitted in front of the touchscreen. In the protocols presented in this Unit the mask provides a row of 5 response locations (4 x 4cm each) spaced 1cm apart at 1.4cm above the floor of the chamber (Figure 2).

Cleaning materials – Disinfectant spray (Distel high level laboratory disinfectant, Tristel Solutions Ltd., Snailwell, UK), paper towels, cleaning brush and filter paper waste tray liners. Touchscreen chambers should be regularly cleaned in accordance with local regulations (e.g. weekly).

Personal protection equipment – FFP2 mask/respirator, disposable gloves and coveralls should be used to minimize animal allergen exposure as mandated by applicable legislation.

Protocol steps

Facility habituation, initial handling and weight regulation

1. If the animals have been transported to the facility in which the procedures are to be conducted allow 7 days acclimatization to the new environment prior to beginning any experimental manipulations. Provide drinking water and standard laboratory rodent food *ad libitum* throughout. To habituate the animals to the relevant experimenter(s), begin basic handling 5 days after arrival in the facility.

2. Individually identify animals using a method approved by local regulations (e.g. ear notching, toe tagging, microchip) and do not handle for the following 24 hours.

3. Weigh mice for 3 consecutive days at approximately the same time and calculate a mean free-feeding weight for each animal.

4. Remove food from all cages, leaving approximately 1 pellet per mouse per cage to begin food restriction.

5. Continue daily weighing, adjusting the number of food pellets provided each day to ensure a gradual weight loss. *Weight loss should be no more than 5% of free feeding weight per day per mouse and should continue until animals reach goal weight (defined as a percentage of their free feeding weight dictated by local regulations – we find that 90% of free feeding weight supports high performance in the paradigms presented in this Unit).*

6. Once animals are at goal weight, adjust daily food provision to halt weight loss and stabilize weight. Continue daily weighing to ensure stable weight levels are maintained.

7. Provide reward (pellets or milkshake) for each cage coincident with daily weighing/feeding for 3 consecutive days. *This is to minimize neophobia to the reward during behavioral training. Pellets should be scattered across the cage, liquid rewards can be provided in a small bowl secured to the cage floor.*

Touchscreen chamber habituation

8. Assign mice to individual behavioral chambers. *These assignments should remain fixed for the duration of behavioral training and testing and should be counter-balanced to ensure even distribution of experimental groups across chambers and testing sessions.*

9. Transfer mice from the housing room to the behavioral testing room and weigh them.

10. Set up the touchscreen testing apparatus, with all input/output devices switched on to ensure animals habituate to any electrical/ultrasonic noise emitted. Fill liquid reward reservoir/pellet hopper to ensure reward-related olfactory cues are present in each chamber.

11. Place approximately 10 reward pellets or 0.2mL liquid reward in the reward collection magazine.

12. Individually transfer animals to assigned chambers and allow them to explore the environment for 20 minutes*. Depending on the specifications of the apparatus, IR beam*

break counts can be used to evaluate locomotor activity, magazine entries and screen touches, and video recordings can be examined for evidence of neophobia/anxiety (e.g. freezing/stereotypies/thigmotaxis).

13. Following completion of the session, remove animals from chambers and return to cages. Inspect magazine/waste tray to confirm reward consumption.

14. Feed mice specified daily number of standard laboratory rodent food pellets upon return to housing room. *Daily food provision may require adjustment to maintain stable weight due to reward consumption.*

15. Repeat daily chamber habituation for at least 2 sessions until performance criterion is reached. *Criterion for advancement to operant response training is consumption of all provided rewards. Ensure procedure is performed at approximately the same time every day.*

Operant response training

16. Commence operant response training the day after habituation performance criterion is reached. Transfer mice to the behavioral testing room and weigh them as previously.

17. Set up the touchscreen testing apparatus for operant response training (see Figure 3 for flowchart summary of the behavioral program). *Animals are permitted a maximum of 60 minutes to complete 30 trials in this training schedule. A single trial consists of presentation of a 4 x 4cm white square stimulus in the central screen response location for 30 seconds. The stimulus is then removed from the screen and a single reward (1 pellet or 20µL liquid) is delivered coincident with magazine illumination and tone delivery (1s, 3kHz). Animals are required to collect reward from the magazine before the next trial will commence after a 5s inter-trial interval (ITI). To facilitate emission of responses to stimuli displayed on the screen, if an animal makes such a response it is reinforced with triple reward delivery.*

18. Following session completion, remove mice from behavioral chambers and return to cages. Inspect magazine/waste tray to confirm reward consumption.

19. Feed mice specified daily number of standard laboratory rodent food pellets upon return to housing room. *Daily food provision may require adjustment to maintain stable weight due to reward consumption.*

20. Repeat daily operant response training at approximately the same time each day until performance criterion is reached. *Criterion for advancement requires animals to complete 30 trials in a single session and consume all earned rewards.*

Fixed ratio training

21. Commence fixed ratio (FR) training the day after operant response training performance criterion is reached. Transfer mice to the behavioral testing room and weigh them as previously.

22. Set up the touchscreen testing apparatus for FR training (see Figure 4 for flowchart summary of the behavioral program). *Animals are permitted a maximum of 60 minutes to complete 30 trials of this training schedule. A single trial consists of presentation of a 4 x 4cm white square stimulus in the central screen response location indefinitely. Animals are required to touch the stimulus, which is then removed from the screen. A single reward is then delivered coincident with magazine illumination and tone delivery (1s, 3kHz). Animals are required to collect reward from the magazine before the next trial will commence after a 4.5s inter-trial interval (ITI). As one operant response is required to elicit a single reward, this schedule is defined as FR 1.*

23. Following session completion, remove mice from behavioral chambers and return to cages. Inspect magazine/waste tray to confirm reward consumption.

24. Feed mice specified daily number of standard laboratory rodent food pellets upon return to housing room. *Daily food provision may require adjustment to maintain stable weight due to reward consumption.*

25. Repeat daily FR 1 training at approximately the same time each day until performance criterion is reached. *Criterion for advancement requires animals to complete 30 trials in a single session and consume all earned rewards.*

26. Upon achievement of FR 1 performance criterion, animals should be advanced to FR 2 training. Repeat all previously specified fixed ratio training steps except substitute the FR 1 behavioral program for the FR 2 program. *The FR 2 program requires emission of two operant screen responses to earn a single reward. Repeated responding is reinforced by brief (500ms) removal of the stimulus following successful screen contact and delivery of a 'chirp' tone (10ms, 3kHz).*

27. Repeat daily FR 2 training at approximately the same time each day until performance criterion is reached. *Criterion for advancement requires animals to complete 30 trials in a single session and consume all earned rewards.*

28. Upon achievement of FR 2 performance criterion, animals should be advanced to FR 3 training. Repeat all previously specified fixed ratio training steps except substitute the FR 2 behavioral program for the FR 3 program, which requires 3 operant responses to be emitted to earn a single reward.

29. Repeat daily FR 3 training at approximately the same time each day until performance criterion is reached. *Criterion for advancement requires animals to complete 30 trials in a single session and consume all earned rewards.*

30. Upon achievement of FR 3 performance criterion, animals should be advanced to FR 5 training. Repeat all previously specified fixed ratio training steps except substitute the FR 3 behavioral program for the FR 5 program which requires 5 operant responses to be emitted to earn a single reward.

31. Repeat daily FR 5 training at approximately the same time each day until performance criterion is reached. *Criterion for advancement requires animals to complete 30 trials in a single session, consume all earned rewards and demonstrate a specificity for the target screen location over the other four never illuminated locations. We typically require a target : blank touch ratio of at least 3:1 (Sharma et al., 2012) for advancement.*

32. Following successful completion of FR 5 training animals can be streamed on to the progressive ratio (PR) (see Basic Protocol 2) or effort-related choice (ERC) (see Basic Protocol 3) paradigms. Food restriction should be maintained throughout.

ALTERNATE PROTOCOL 1

Initial handling, food restriction and operant chamber acclimatization adapted for progressive neurodegenerative mouse models

The procedure presented in Basic Protocol 1 is highly effective for the rapid training of wild type mice to the required FR 5 performance standard making it ideal for use in systemic/central pharmacology studies and experiments involving post response acquisition surgical manipulations. However, there are circumstances in which this procedure may impede

subsequent behavioral evaluation as the increase in response requirement between the FR 1 and FR 5 training programs can result in prolonged performance suppression and the FR 5 target : blank performance criterion may be excessively stringent for some animal groups. In particular, transgenic models of neurodegenerative disease including Alzheimer's and Huntington's and also aged mice may be excessively challenged by Basic Protocol 1 and may not reach PR or ERC evaluation before development of excessive pathology. We therefore recommend the following Alternate Protocol 1 be used in cases in which a given genetic/surgical/pharmacological manipulation may compromise behavioral performance.

Materials

Husbandry – In some cases it may be necessary to provide standard laboratory chow that has been moistened with water to sustain the body weight of manipulated mice on a daily basis following commencement of food restriction.

Rewards – For behaviorally compromised mice we recommend use of liquid reward equipped chambers. Liquid reward consumption is not compromised by potential issues related to experimental manipulations such as dry mouth or compromised chewing.

Protocol steps

All Protocol steps are identical to those detailed in Basic Protocol 1 with the following exceptions:

1. The number of trials required in the FR 1, FR 2 and FR 3 programs should be adjusted such that the total number of touches required for completion is the same across all schedules. Criterion for advancement should be modified to reflect the adjusted trial number and still require all earned rewards to be consumed. *While the number of trials implemented should be determined on an experiment-to-experiment basis, we have found sequences such as: FR 1 = 12 trials; FR 2 = 6 trials; FR 3 = 4 trials (all requiring 12 screen touches in total) to be effective.*

2. Following completion of FR 3 training, mice should be streamed either to the modified PR protocol (see Alternate Protocol 2) or the standard ERC protocol (see Basic Protocol 3). Food restriction should be maintained throughout.

BASIC PROTOCOL 2

Assessment of mouse motivation using the touchscreen progressive ratio (PR) task

Following successful training of the touchscreen operant response (see Basic Protocol 1) mice can be transferred to the PR task (Hodos, 1961). This paradigm provides a method to assess the motivation of an animal to obtain a reward by measuring the amount of physical effort that animal is willing to expend to gain access to that reward (Markou et al., 2013). This is achieved by increasing the number of touchscreen responses required to earn a single reward across trials until responding ceases (Heath et al., In press). This Basic Protocol presents an experimental design appropriate for characterizing animals expressing stable (typically non-degenerative) genetic modifications or surgical manipulations such as focal lesions.

Materials

No additional materials beyond those specified in Basic Protocol 1 are required for this Protocol.

Protocol steps

1. Commence progressive ratio (PR) training the day after FR performance criterion is reached (see Basic Protocol 1). Transfer mice to the behavioral testing room and weigh them as previously.

2. Set up the touchscreen testing apparatus for PR training (see Figure 5 for flowchart behavioral program summary). *Animals are permitted a maximum of 60 minutes per session to complete as many trials as possible. The first trial of all PR sessions requires a single operant screen response after which a single reward is delivered coincident with magazine illumination and tone delivery (1s, 3kHz). Animals are required to collect reward from the magazine before the next trial will commence after a 4.5s inter-trial interval (ITI). The response requirement is increased in all subsequent trials according to a linear ramp with repeated touches supported by brief (500ms) removal of the screen stimulus following successful screen contact and delivery of a 'chirp' tone (10ms, 3kHz). In wild type mice we have found a linear +4 (PR 4) schedule (i.e. 1, 5, 9, 13 touches per trial) to provide stable performance levels that can be bi-directionally modulated by systemic pharmacological manipulations* (Heath et al., In press)*. If animals do not emit a screen touch or magazine entry following reward delivery for 300s the PR schedule ends.*

3. Following session completion, either due to reaching the maximum time limit or inactivity time out, remove mice from behavioral chambers and return to cages. Inspect magazine/waste tray to confirm reward consumption.

4. Feed mice specified daily number of standard laboratory rodent food pellets upon return to housing room. *Daily food provision may require adjustment to maintain stable weight due to reward consumption.*

5. Repeat PR training on the same schedule for a further 2 consecutive sessions. *If the acute effects of a pharmacological manipulation on task performance are to be investigated, we recommend continuing PR training and administering injections after 3 consecutive sessions of training on this schedule.*

6. Following completion of 3 consecutive PR sessions, return mice to the FR 5 schedule (see Basic Protocol 1) for 3 consecutive sessions. *This manipulation is used to minimize potential performance degradation induced by repeated exposure to the PR schedule.*

7. Repeat PR training on the same schedule applied in Step 5 for a further 3 consecutive sessions.

8. Return mice to the FR 5 schedule for 3 consecutive sessions.

9. Assess mean performance across both blocks of PR sessions. *Variables commonly evaluated include: breakpoint (defined as the number of screen responses emitted on the last trial successfully completed before session end/time out), total number of correct screen touches, reward collection latency, post-reinforcement pause (defined as the time interval between removal of the head from the magazine following reward collection and the first screen touch of the next trial) and the rate of arena and magazine IR beam breaks.*

10. Repeat PR training for a further 3 consecutive sessions using a higher demand linear ramp. *We routinely assess the performance of mice across multiple evenly spaced linear ramps (e.g. PR 4, PR 8, PR 12) to evaluate the consistency of any performance-related phenotypes detected.*

11. Return mice to the FR 5 schedule for 3 consecutive sessions.

12. Repeat PR training on the same schedule applied in Step 10 for a further 3 consecutive sessions

13. Analyze data with variables detailed in Step 9.

ALTERNATE PROTOCOL 2

Assessment of motivation using the touchscreen progressive ratio (PR) task adapted for mouse models of progressive neurodegenerative disease

The variant of the PR assessment detailed in Basic Protocol 2 may be inappropriate for certain mouse models of neurodegenerative disease. In particular, mice with rapidly progressing pathology leading to motoric compromise may exhibit significant performance suppression or inconsistency through repeated PR – FR – PR transitions or perform at behavioral floor levels by increasing linear ramp gradients. We suggest that such animals (which are likely to have been trained initially according to Alternate Protocol 1) be streamed to this variant of the PR assessment.

Materials

No additional materials are required for this Protocol.

Protocol steps

1. Follow Steps 1 – 4 of Basic Protocol 2. *We recommend assessing potentially compromised mice initially on a linear +1 (PR 1) ramp to ensure performance is maximized.*

2. Analyze data with variables detailed in Step 9 of Basic Protocol 2.

3. Repeat daily PR sessions until performance stabilizes. *We recommend a stability criterion of less than 10% variability in breakpoint across 2 consecutive sessions.*

4. Repeat Steps 1 – 3 of this Protocol using a linear +2 (PR 2) ramp. *If performance is suppressed to floor levels and the mice concerned are to be used in a pharmacological/surgical study, the PR ramp should be returned to the previous PR 1 schedule and daily sessions should be conducted until performance is restored.*

5. Repeat Steps 1 – 3 of this Protocol using a linear +4 (PR 4) ramp. *If performance is suppressed to floor levels and the mice concerned are to be used in a pharmacological/surgical study, the PR ramp should be returned to the previous PR 2 schedule and daily sessions should be conducted until performance is restored.*

BASIC PROTOCOL 3

Evaluation of reward-related decision making in the mouse using the touchscreen version of the effort-related choice (ERC) task

Following successful training of the touchscreen operant response (see Basic Protocol 1) and/or assessment on the PR task (see Basic Protocol 2), animals can be evaluated in the ERC paradigm (Salamone et al., 1991). This task assesses reward-related decision making by allowing animals to make a choice between emitting a fixed number of operant screen responses to obtain a single highly palatable reward or exerting no effort to consume a freely available but relatively less palatable food present in the behavioral arena (Heath et al., In press).

Materials

No additional materials beyond those specified in Basic Protocol 1 are required for this Protocol.

Protocol steps

1. Commence effort-related choice (ERC) training the day after FR performance criterion is reached (see Basic Protocol 1) or PR assessment is completed (see Basic Protocol 2). Transfer mice to the behavioral testing room and weigh them as previously.

2. Put clean filter paper liners in the waste collection tray beneath the arena floor of all chambers to be used for the ERC task.

3. For each chamber weigh three standard laboratory rodent food pellets and scatter them randomly across the arena floor.

4. Set up the touchscreen testing apparatus for the operant component of the ERC task (see Figure 6 for flowchart behavioral program summary). *Animals are permitted a maximum of 60 minutes to complete 30 operant trials in this schedule, which is identical to the FR schedule presented in Basic Protocol 1. We have found that relatively strenuous schedules such as FR 8 or FR 16 provide suitable performance levels for initial task training.*

5. Following session completion, either due to reaching the maximum time limit or consumption of 30 rewards, remove mice from behavioral chambers and return to cages.

6. Collect and weigh any remaining food pellets/pellet fragments from the arena floor/waste collection tray.

7. Feed mice specified daily number of standard laboratory rodent food pellets upon return to housing room. *Daily food provision may require adjustment to maintain stable weight due to reward/standard food consumption as part of the ERC protocol.*

8. Analyze data. *Variables commonly evaluated include number of operant trials completed/number of rewards earned, total number of screen touches emitted and weight of food pellets consumed*. *Lower response requirements may necessitate removal of animals from chambers before 60 minutes have elapsed and therefore a rate correction may have to be applied during data analysis to account for differing session lengths.*

9. Repeat daily ERC sessions using the same operant work requirement for 8 consecutive days.

10. Analyze data based on mean animal performance across all sessions.

11. Repeat Steps 1 – 10 with an elevated work requirement (e.g. transition from FR 8 to FR 16). *In the face of the increasing operant work requirement, wild type mice should gradually alter their behavioral profile to consume progressively more of the freely available standard laboratory rodent food and emit fewer operant responses* (Heath et al., In press). *We routinely screen performance across three evenly spaced operant work requirements to evaluate consistency of decision-making and determine if animals respond predictably to elevation of the operant work requirement.*

REAGENTS AND SOLUTIONS

No additional reagents or solutions are required for any of the Protocols presented in this Unit.

COMMENTARY

Background Information

The operant touchscreen apparatus is becoming increasingly widely used in studies that involve examination of the effects of a given pharmacological, genetic or surgical manipulation on rodent cognition. This apparatus provides a versatile, high throughput and standardized

approach to the assessment of a broad range of cognitive domains, combined with minimal experimenter-subject contact, elimination of subjective bias in data collection and analysis and offers higher face validity to contemporary computer-based human cognitive assessments than other rodent behavioral assessment techniques (e.g. maze-based tasks) (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013; Bussey et al., 2012).

Extant paradigms for the touchscreen platform allow examination of a number of psychological constructs including attention, working memory, compulsivity, impulsivity and perceptual discrimination as part of an integrated battery approach (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). Here we provide protocols for measuring motivation and reward (value)-related decision-making in this apparatus based on validated studies from our laboratory (Heath et al., In press). Such a capacity is of substantial benefit in that it allows these constructs to be examined as part of the battery, thereby increasing the breadth of the phenotypic characterization possible in individual animals. Similarly, it enables the inherent advantages of the touchscreen platform to be leveraged in independent studies of these constructs.

The capability to assess motivation for reward in the touchscreen apparatus is also of value due to the exclusive use of appetitive reinforcement in all of the available cognitive assessment paradigms (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). While highly beneficial from the perspective of animal welfare and being critical in facilitating the battery approach in that individual animals can be tested on multiple behavioral paradigms within the same apparatus, the use of appetitive reinforcement requires that all experimental groups be equally motivated to earn and consume reward (Bussey et al., 2012). Between group differences in motivation for reward could potentially confound the interpretation of any performance differences detected in a cognitive assessment task unless appropriately accounted for in experimental design (Bussey et al., 2012), which this capability permits. Significantly, integration of the motivational assessment into the touchscreen battery allows this construct to be measured in the same apparatus and requires animals to emit the same operant response as used in the cognitive assessment tasks, thereby increasing the robustness of any study in which it is concluded that a difference in cognitive task performance is not due to a between group difference in motivation for reward.

The development of the Protocols described in this Unit therefore substantially enhances the breadth of the behavioral assessment possible with the rodent touchscreen apparatus.

The PR task (Hodos, 1961) is used to assess motivation by evaluating the capacity of a rodent to maintain operant responding despite regular increases in the number of responses

required to earn a reward (Markou et al., 2013). This paradigm has been used extensively in laboratory rats and mice to assess changes in motivation due to transgenic modification (Young et al., 2011; Drew et al., 2007), surgical manipulation (Gourley et al., 2010; Trifilieff et al., 2013) and both acute (Bensadoun et al., 2004; Aberman et al., 1998) and chronic pharmacological treatments (Gourley et al., 2008; Olausson et al., 2013; Aberman et al., 1998). While both rodent nosepoke and lever mechanisms have been used as response manipulanda in the PR task previously, until the optimization of the Protocols presented in the Unit, the viability of the touchscreen to support the type of sustained, repetitive responding required for this paradigm was unknown (Heath et al., In press).

The ERC task (Salamone et al., 1991) is used to evaluate reward-related decision making in rodents by establishing an outcome choice scenario in which animals can dynamically perform cost/benefit calculations to select between expending no effort and consuming freely available standard laboratory rodent food or emitting operant responses to obtain access to a relatively more palatable food reward (Salamone et al., 1991; Markou et al., 2013). The ERC task has been used previously to assess the effects of a variety of pharmacological (Nunes et al., 2013a; Salamone et al., 1991; Nunes et al., 2013b), genetic (Pardo et al., 2012; Ward et al., 2012) and surgical manipulations (Salamone et al., 1991) on choice behavior (Salamone et al., 2012). The ERC task has also been used to investigate potential mechanisms underlying motivational differences detected by the PR task, therefore providing a powerful complement to the other task presented in this Unit (Ward et al., 2012; Drew et al., 2007). In addition, while a version of this task based on the T-maze does exist (Pardo et al., 2012), we believe that there are a number of advantages of a touchscreen-based version (Heath et al., In press) as previously outlined.

Critical Parameters

Several factors must be considered when designing and implementing the protocols presented in the Unit. In particular, consideration of the capacity of the animals being investigated for operant behavior is essential and may, for example, dictate whether use of Alternate Protocols 1 and 2 instead of Basic Protocols 1 and 2 is appropriate.

As noted, this is particularly important when evaluating rodent models of neurodegenerative disease that may rapidly become motorically compromised, thereby confounding interpretation of any observed differences in behavioral performance. Such studies may therefore necessitate the use of less effortful behavioral schedules, less stringent performance criteria and reduced trial number per day requirements to ensure evaluations can

be conducted. The suitability of these parameters should be regularly evaluated during the course of a study involving these paradigms, particularly in the initial operant response and FR training as it is critical that no particular group (either experimentally manipulated or control) receives substantially more or less exposure to the training schedules and is consequently over- or under-trained when transferred to the PR or ERC schedules. Alleviation of this may require matching the number of trials performed by each group involved in a study during the initial operant response and FR training paradigms.

The parameters of studies involving aged animals and/or models of aging that may have a shortened lifespan and/or compromised operant behavior capacity should similarly be modified to compensate for the potentially diminished performance and reduced longevity in the manipulated group. As age may have profound effects on performance in these tasks, we also recommend that variability in this parameter both within and between groups is kept to a minimum wherever possible.

While it is not always feasible to select between use of solid and liquid rewards due to equipment specifications and availability, we recommend selection of the latter as far as possible. Although we have not conducted a rigorous comparative evaluation of the two reward types, we suggest that liquid may be more efficacious in studies involving animals which could be behaviorally compromised as it alleviates performance suppression issues related to dry mouth or difficulties in chewing solid materials.

All of the paradigms presented in this Unit are particularly sensitive to changes in animal body weight due to their dependence on consumption of palatable gustatory rewards. The importance of body weight maintenance for stable behavioral performance must be emphasized. Animals should be weighed daily at the same time and measures such as provision of moistened food and briefly isolating mice during feeding to ensure equal distribution of daily food provisions should be implemented if prodigious changes in weight are detected. Similarly, body weights should be rigorously evaluated during PR and ERC studies as between group differences in this parameter could correlate with between group differences in task performance in these paradigms.

Troubleshooting

A number of common issues related to the conduct of the Protocols described in this Unit are summarized in Table 1. The performance of mice in these tasks is particularly sensitive to body weight changes and it is critical that food restriction and weight maintenance be strictly controlled to ensure stability. However, despite rigorous weight control a small proportion of

animals will occasionally be unable to complete training. These animals should be excluded after a fixed number of training sessions (based on typical group performance). In our experience a drop-out rate of approximately 10% is reasonable in the majority of studies.

To ensure minimal hardware-related disruptions, it is also advisable to regularly test all components of the touchscreen chambers (e.g. at least weekly) and to maintain a supply of spare components for the equipment. In particular, IR activity beam assemblies, touchscreens, connector cables and pellet dispensers, which in our experience are particularly susceptible to failure, should be readily available.

Anticipated Results

The chamber habituation sessions presented in Basic Protocol 1 can provide arena, magazine and screen IR beam break counts to allow assessment of non-specific locomotor activity. This can indicate systematic between group differences prior to operant training which may impact task performance and paradigm parameter adjustment may be required to compensate. Inspection of videos taken from overhead arena cameras may also be used to assess animal groups for evidence of differences in freezing, stereotyped or thigmotaxic behaviors.

Performance in the initial operant response and FR training paradigms is typically presented as the number of trials completed in each session conducted. We generally find that animals learn the simple operant response required and reach the maximum number of trials specified very rapidly (Heath et al., In press). The number of blank touches (i.e. responses to the 4 never illuminated touchscreen response locations) is also recorded and the ratio of target location : blank location touches should be calculated (in the case of studies following Basic Protocol 1). In wild type mice training on the FR 5 schedule we find that this ratio rapidly increases and usually exceeds the 3:1 target within 3 – 5 sessions (Heath et al., In press). Chamber IR beam break rates generated during these sessions can also be assessed for general locomotor activity differences.

PR paradigm performance is routinely presented in the form of a comparison of breakpoints derived from different experimental groups and/or under different conditions of pharmacological challenge. We also recommend evaluating the total number of target location touches emitted per session as breakpoint only captures the number of responses emitted to earn the last reward of a session. While two animals may yield identical breakpoint values, it is possible that one individual may have emitted a vastly greater number of screen touches while progressing towards completing the next trial (which therefore suggests substantially increased

motivation for reward) which is not expressed by this parameter. In wild type mice we find that breakpoint performance stability is rapidly achieved (within 7 sessions of exposure to the PR paradigm) (see Figure 7A) and can be bi-directionally modulated by acute systemic administration of amphetamine (see Figure 7B) or sulpiride (see Figure 7C). Furthermore, when conducting studies in which the PR linear ramp is progressively elevated (PR 2, PR 4, PR 8 etc.) we find that wild type mice exhibit progressive increases in breakpoint and decreases in the total number of correct touches emitted per session and trials completed as the ramp is raised.

Results of studies using the ERC paradigm are typically presented as a comparison of the number of operant trials completed and/or the volume or weight of reward earned versus the weight of standard laboratory rodent food consumed as a function of the operant work requirement used and/or experimental manipulation (e.g. pharmacological challenge) applied. In wild type mice in baseline conditions we find that as the operant work requirement is progressively increased the behavioral profile shifts from favoring operant responding toward consumption of freely available standard food (see Figure 7D, E).

Time Considerations

All of the Protocols presented in this Unit are rapid to perform. Following initial acclimatization to the housing facility (7 days), initial handling, food restriction and reward habituation should take between 7 and 10 days. Wild type mice require between 1 and 3 days to reach operant response training performance criterion and completion of FR 1, 2, 3 and 5 training typically requires a total of 6 to 10 days.

Following FR training, each PR work requirement to be examined requires 9 sessions (2 blocks of 3 PR sessions with an interstitial block of 3 FR 5 sessions). If the acute effects of a pharmacological manipulation on PR performance are to be investigated, we recommend allowing animals to perform 3 consecutive PR sessions prior to drug administration to ensure stable performance. Regarding the ERC paradigm, as noted in Basic Protocol 3, we typically assess performance across 3 operant work requirements with 8 consecutive sessions at each requirement, requiring a total of 24 sessions. Additional sessions may be required in studies involving neurodegenerative disease models or otherwise behaviorally compromised animals.

Regarding daily time demands, animal handling/weighing may take up to 60 minutes (dependent on experimenter experience and number of animals) and preparation and testing of touchscreen equipment a further 60 minutes. Each behavioral session (including transfer of mice to and from touchscreen chambers) may require 70 minutes, although we often find that animals do not reach the 60 minute session maximum, considerably reducing this requirement.

Animal feeding and cleaning/shut down of touchscreen equipment may require up to 90 minutes (depending on the number of animals and number of chambers used) and daily data analysis may require 30 – 60 minutes (depending on training stage and number of animals involved).

We routinely train mice on a daily basis 7 days per week to ensure rapid progression and stable behavioral performance. However, we have found that these protocols are relatively resistant to disruption induced by a single 'rest' day in any given week so performance should not be adversely affected by training 6 days per week. We strongly recommend timing such 'rest' days not to coincide with task critical events such as the introduction of a new PR or ERC work requirement.

ACKNOWLEDGEMENT

The protocols presented in this Unit were developed and optimized as part of a research program funded by Wellcome Trust grant 089703/Z/09/Z awarded to TJB and LMS. TJB and LMS also received funding from the Innovative Medicine Initiative Joint Undertaking under grant agreement n° 115008 of which resources are composed of EFPIA in-kind contribution and financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013). TJB and LMS consult for Campden Instruments Ltd.

LITERATURE CITED

Aberman, J. E., Ward, S. J., and Salamone, J. D. 1998. Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. *Pharmacology, Biochemistry, and Behavior* 61:341–348.

Beeler, J. A., Prendergast, B., and Zhuang, X. 2006. Low amplitude entrainment of mice and the impact of circadian phase on behavior tests. *Physiology & Behavior* 87:870–880.

Bensadoun, J.-C., Brooks, S. P., and Dunnett, S. B. 2004. Free operant and discrete trial performance of mice in the nine-hole box apparatus: validation using amphetamine and scopolamine. *Psychopharmacology* 174:396–405.

Bussey, T. J., Holmes, A., Lyon, L., Mar, A. C., McAllister, K. A. L., Nithianantharajah, J., Oomen, C. A., and Saksida, L. M. 2012. New translational assays for preclinical modelling of cognition in

schizophrenia: the touchscreen testing method for mice and rats. *Neuropharmacology* 62:1191–1203.

Chaudhury, D., and Colwell, C. S. 2002. Circadian modulation of learning and memory in fear-conditioned mice. *Behavioural Brain Research* 133:95–108.

Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H., and Bussey, T. J. 2010. Running enhances spatial pattern separation in mice. *Proceedings of the National Academy of Sciences of the United States of America* 107:2367–2372.

Drew, M. R., Simpson, E. H., Kellendonk, C., Herzberg, W. G., Lipatova, O., Fairhurst, S., Kandel, E. R., Malapani, C., and Balsam, P. D. 2007. Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. *The Journal of neuroscience: the official journal of the Society for Neuroscience* 27:7731–7739.

Frick, K. M., and Berger-Sweeney, J. 2001. Spatial reference memory and neocortical neurochemistry vary with the estrous cycle in C57BL/6 mice. *Behavioral Neuroscience* 115:229–237.

Gourley, S. L., Lee, A. S., Howell, J. L., Pittenger, C., and Taylor, J. R. 2010. Dissociable regulation of instrumental action within mouse prefrontal cortex. *The European Journal of Neuroscience* 32:1726–1734.

Gourley, S. L., Wu, F. J., Kiraly, D. D., Ploski, J. E., Kedves, A. T., Duman, R. S., and Taylor, J. R. 2008. Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. *Biological psychiatry* 63:353–359.

Heath, C. J., Bussey, T. J., and Saksida, L. M. In press. Motivational assessment of mice using the touchscreen operant testing system: effects of dopaminergic drugs. *Psychopharmacology*.

Hodos, W. 1961. Progressive ratio as a measure of reward strength. *Science (New York, N.Y.)* 134:943–944.

Horner, A. E., Heath, C. J., Hvoslef-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R. O., Alsiö, J., Oomen, C. A., Holmes, A., Saksida, L. M., et al. 2013. The touchscreen operant platform for testing learning and memory in rats and mice. *Nature protocols* 8:1961–1984.

Mar, A. C., Horner, A. E., Nilsson, S. R. O., Alsiö, J., Kent, B. A., Kim, C. H., Holmes, A., Saksida, L. M., and Bussey, T. J. 2013. The touchscreen operant platform for assessing executive function in rats and mice. *Nature protocols* 8:1985–2005.

Markou, A., Salamone, J. D., Bussey, T. J., Mar, A. C., Brunner, D., Gilmour, G., and Balsam, P. 2013. Measuring reinforcement learning and motivation constructs in experimental animals: Relevance to the negative symptoms of schizophrenia. *Neuroscience and biobehavioral reviews* 37:2149–2165.

Meziane, H., Ouagazzal, A.-M., Aubert, L., Wietrzych, M., and Krezel, W. 2007. Estrous cycle effects on behavior of C57BL/6J and BALB/cByJ female mice: implications for phenotyping strategies. *Genes, Brain, and Behavior* 6:192–200.

Nunes, E. J., Randall, P. A., Hart, E. E., Freeland, C., Yohn, S. E., Baqi, Y., Müller, C. E., López-Cruz, L., Correa, M., and Salamone, J. D. 2013a. Effort-Related Motivational Effects of the VMAT-2 Inhibitor Tetrabenazine: Implications for Animal Models of the Motivational Symptoms of Depression. *The Journal of neuroscience: the official journal of the Society for Neuroscience* 33:19120–19130.

Nunes, E. J., Randall, P. A., Podurgiel, S., Correa, M., and Salamone, J. D. 2013b. Nucleus accumbens neurotransmission and effort-related choice behavior in food motivation: effects of drugs acting on dopamine, adenosine, and muscarinic acetylcholine receptors. *Neuroscience and biobehavioral reviews* 37:2015–2025.

Olausson, P., Kiraly, D. D., Gourley, S. L., and Taylor, J. R. 2013. Persistent effects of prior chronic exposure to corticosterone on reward-related learning and motivation in rodents. *Psychopharmacology* 225:569–577.

Oomen, C. A., Hvoslef-Eide, M., Heath, C. J., Mar, A. C., Horner, A. E., Bussey, T. J., and Saksida, L. M. 2013. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. *Nature protocols* 8:2006–2021.

Pardo, M., Lopez-Cruz, L., Valverde, O., Ledent, C., Baqi, Y., Müller, C. E., Salamone, J. D., and Correa, M. 2012. Adenosine A2A receptor antagonism and genetic deletion attenuate the effects of dopamine D2 antagonism on effort-based decision making in mice. *Neuropharmacology* 62:2068–2077.

Roedel, A., Storch, C., Holsboer, F., and Ohl, F. 2006. Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. *Laboratory Animals* 40:371–381.

Salamone, J. D., Correa, M., Nunes, E. J., Randall, P. A., and Pardo, M. 2012. The behavioral pharmacology of effort-related choice behavior: dopamine, adenosine and beyond. *Journal of the Experimental Analysis of Behavior* 97:125–146.

Salamone, J. D., Steinpreis, R. E., McCullough, L. D., Smith, P., Grebel, D., and Mahan, K. 1991. Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology* 104:515–521.

Sharma, S., Hryhorczuk, C., and Fulton, S. 2012. Progressive-ratio responding for palatable high-fat and high-sugar food in mice. *Journal of Visualized Experiments: JoVE*:e3754.

Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R. D., Taylor, K. M., Martinez, D., Moore, H., Balsam, P. D., Simpson, E. H., et al. 2013. Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. *Molecular Psychiatry* 18:1025–1033.

Ward, R. D., Simpson, E. H., Richards, V. L., Deo, G., Taylor, K., Glendinning, J. I., Kandel, E. R., and Balsam, P. D. 2012. Dissociation of hedonic reaction to reward and incentive motivation in an animal model of the negative symptoms of schizophrenia. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 37:1699–1707.

Wolf, J. E., Urbano, C. M., Ruprecht, C. M., and Leising, K. J. 2014. Need to train your rat? There is an App for that: a touchscreen behavioral evaluation system. *Behavior Research Methods* 46:206–214.

Young, J. W., Meves, J. M., Tarantino, I. S., Caldwell, S., and Geyer, M. A. 2011. Delayed procedural learning in α7-nicotinic acetylcholine receptor knockout mice. *Genes, Brain, and Behavior* 10:720–733.

FIGURE LEGENDS

Figure 1: Photograph annotated to highlight the key features of a standard Campden Instruments mouse touchscreen chamber. A: Trapezoidal behavioral arena. B: Touchscreen. C: Reward collection magazine. D: Transparent arena lid to allow camera observation of performance. E: Arena floor. F: Sound attenuating chamber. G: Fan ventilator. H: Camera, tone/click generator, house light and pellet dispenser assembly. I: Liquid reward pump. J: IR activity beam assembly.

Figure 2: Overhead photograph illustrating five location touchscreen mask configuration used in all of the Protocols presented in this Unit.

Figure 3: Flowchart summary of operant response training protocol. The target stimulus is displayed in the central touchscreen response window. After 30s or if the mouse touches the

stimulus, it is removed and reward is delivered (triple delivery if operant response emitted). Following reward collection and ITI the target stimulus is again displayed and the next trial begins.

Figure 4: Flowchart summary of FR protocol. The target stimulus is displayed in the central touchscreen response window. Stimulus display is maintained until the animal emits an operant response in that window. In the FR 1 schedule a screen response elicits reward delivery; in all other FR schedules the stimulus is re-displayed and further operant responses are required for reward delivery. Following reward collection and ITI the next trial begins with stimulus display.

Figure 5: Flowchart summary of PR protocol. The target stimulus is displayed in the central touchscreen response window. Stimulus display is maintained until the animal emits an operant response in that window. Upon completion of the response requirement for a given trial, reward is delivered. Following reward collection and ITI the response requirement is incremented according to the pre-specified linear ramp and applied to the following trial, which begins with stimulus display.

Figure 6: Flowchart summary of the ERC protocol. Animals are free to select dynamically either the 'high effort – high value' behavioral profile which follows the same event sequence as the FR schedule to earn access to reward pellets/milkshake or the 'low effort – low value' profile which permits consumption of standard laboratory rodent food for the expenditure of minimal effort.

Figure 7: Representative data collected from adult male C57Bl/6 mice performing in the touchscreen PR and ERC paradigms (Heath et al., In press). A: Stabilization of linear PR 4 ramp performance within 3 sessions. Main effect of session: $F(2.63,44.68) = 7.34$; $p = 0.01$. Post-hoc analysis indicates no significant breakpoint differences after session 3 ($p = 1.000$ in all cases). B: Acute systemic administration of amphetamine significantly increases linear PR 4 breakpoint. Main effect of drug: $F(2,34) = 27.40$; $p<0.001$. Post-hoc analysis indicates a significant increase versus vehicle following 1mg/kg administration ($p<0.001$). C: Acute systemic administration of sulpiride significantly decreases linear PR 4 breakpoint. Main effect of drug: $F(2,34) = 3.98$; $p = 0.028$. Post-hoc analysis indicates a significant decrease versus vehicle following 50mg/kg administration ($p = 0.009$). D: Increasing ERC operant work requirement results in increased consumption of freely available standard rodent chow. Main effect of work requirement: $F(2,30) = 57.138$; $p<0.001$. Post hoc-analysis indicates significant increases between each work requirement ($p<0.001$ in all cases). E: Increasing ERC operant work requirement results in decreased milkshake volume earned. Main effect of work requirement: $F(1.113,16.702) = 133.187$; $p<0.001$. Post-hoc analysis indicates significant decreases between each work requirement ($p<0.001$ in all cases).

TABLES

Table 1: Troubleshooting summary

Problem	Likely Causes	Solution
Poor performance during training (trial incompletion or failure to consume all rewards earned).	Insufficiently food restricted.	Reduce food availability until animals are between 85-90% of their free-feeding weight (in accordance with local welfare regulations).
	Insufficient habituation to reward.	Present additional reward in home cages.
	Aversion to touchscreen, mask or reward location.	Additional chamber habituation sessions.
	Stressor in home environment.	Frequently check housing rooms for stressors (e.g.: excessive noise, fighting in cages, unusual scent). Consider animal separation or relocation.
One group in a between-group design is progressing through training at a faster rate than the other.	One group exhibits a task-relevant impairment or facilitation as a result of a genetic alteration or other treatment.	Reduce trial cap on fixed ratio schedule in order to 1) facilitate progression in the slower group, 2) prevent the faster group from greater exposure to the touch-reward contingency and satiety.
	Between-group difference in body weight.	Ensure that absolute and % of free-feeding body weights are as close as possible. Consider temporary single-housing during feeding.
A well trained animal exhibits	Reward pellet dispenser failure	Clear blockage in pellet

unexpectedly reduced trial completion during a session.	or blockage in liquid reward delivery tube.	dispenser mechanism and test to confirm operating normally. Clear the reward delivery tube and run liquid reward into the magazine aperture. Carefully assess the degree to which the animal subsequently responds. Ensure reward delivery tubes are thoroughly cleaned at the end of each day with hot water in order to prevent blockages.
	Touchscreen error.	Check for stimulus presentation and touch sensitivity. Restart system and check all physical connections. Run a test program to ensure touchscreen is functional.
	Control system failure.	Check software and hardware. Check physical connections and restart controlling system and touchscreen.
Unexpectedly excessive or low number of beam breaks during a session.	Infrared beam fault.	Check infrared beam status with test program. Ensure that infrared beams are clean and clear of obstruction. Replace faulty hardware.
	Excessive grooming at infrared beam location.	Observe and take into consideration during data analysis (stereotypies).

FIGURES

Figure 1:

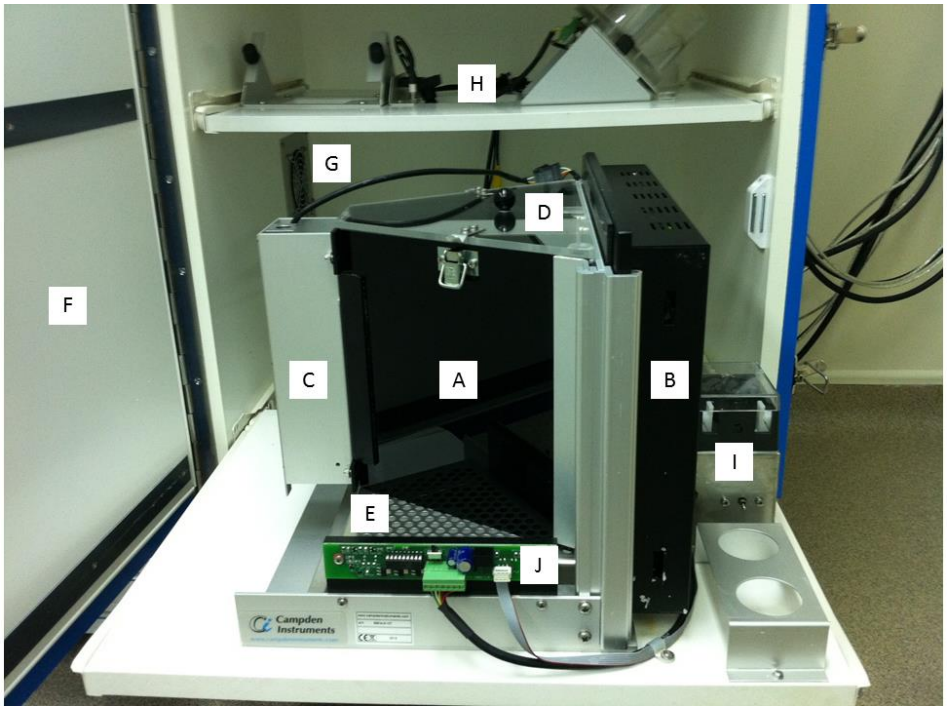


Figure 2:

Figure 3:

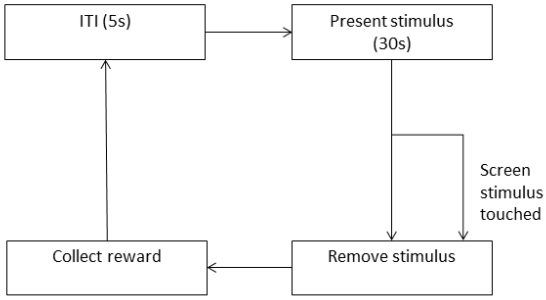


Figure 4:

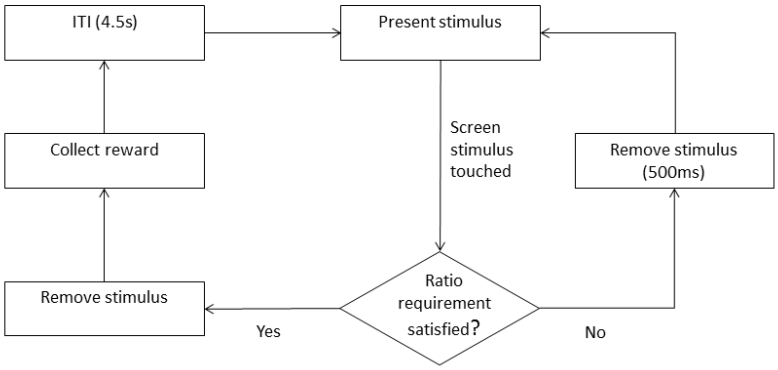


Figure 5:

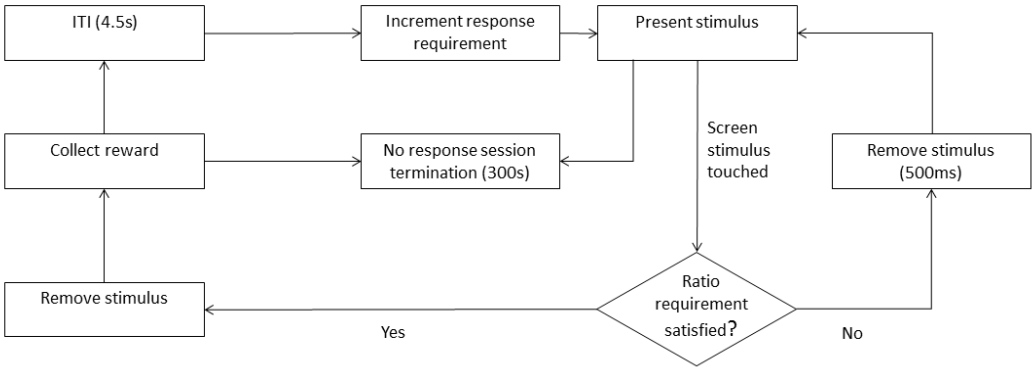


Figure 6:

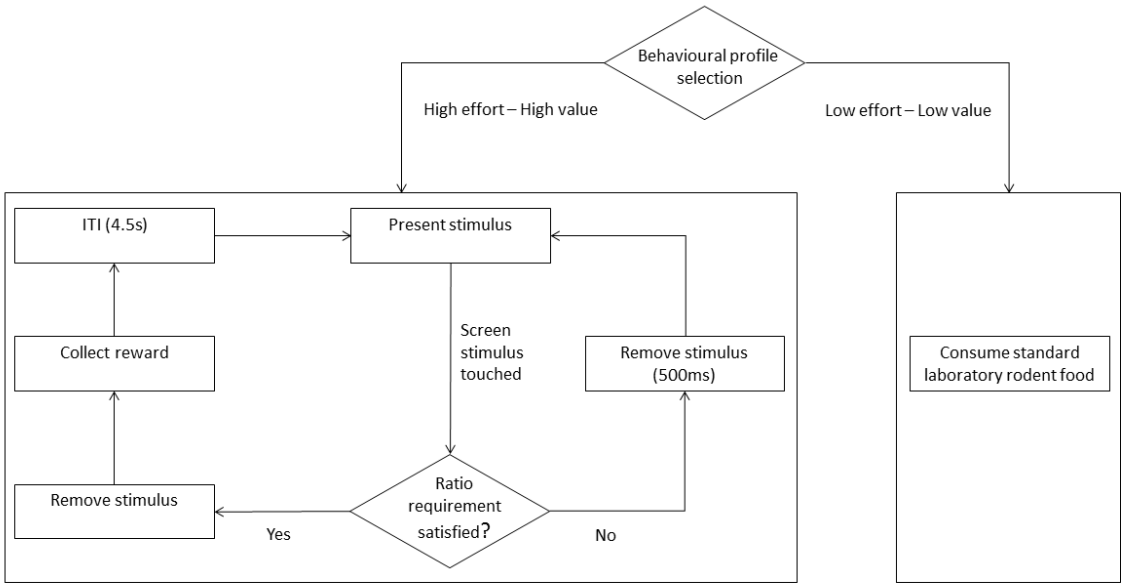
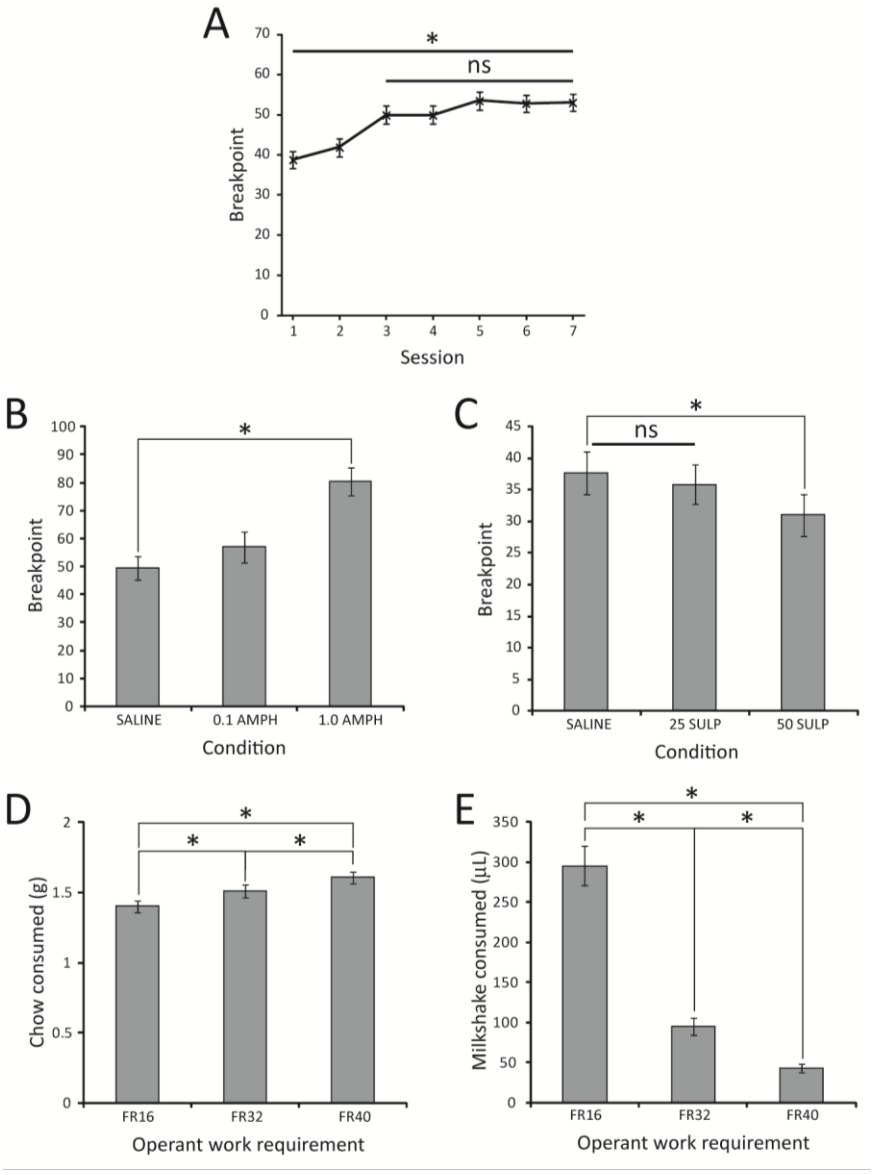

Behavioural profile selection
High effort – High value
Low effort – Low value
ITI (4.5s)
Present stimulus
Collect reward
Screen stimulus touched
Remove stimulus (500ms)
Consume standard laboratory rodent food
Remove stimulus
Ratio requirement satisfied?
Yes
No

Figure 7:


A
*
ns
Breakpoint
Session
B
*
Breakpoint
SALINE
0.1 AMPH
1.0 AMPH
Condition
C
*
ns
Breakpoint
SALINE
25 SULP
50 SULP
Condition
D
*
*
*
Chow consumed (g)
FR16
FR32
FR40
Operant work requirement
E
*
*
*
Milkshake consumed (µL)
FR16
FR32
FR40
Operant work requirement